

## REMARKS

Independent claims 1, 15 and 16 were canceled. Withdrawn claims 10 and 11 were canceled. Claims 2, 3, 4, and 7 were amended. New claims 17-19 were added.

### Information Disclosure Statement

Translations or an English language equivalent of Japanese Patents Nos. 2003-207507, 7-191033A and 63-184063 were provided in the initial filing of the Information Disclosure Statement (IDS). The translation of Japanese patent no. 2003-207507 is on pages 18-42 of the document filed with the IDS in the USPTO on July 27, 2006. The translation of Japanese patent no. 7-191033A is on pages 6-16 of the document submitted to the USPTO. The European equivalent application (EP 0 268 773 A1) to Japanese patent no. 63-184063 was provided starting on page 8 of the submitted document. Since an English language translation or English language equivalent were provided for all three Japanese patent documents, it is respectfully requested that the Information Disclosure Statement of July 27, 2006 be considered and entered into this application.

### Rejection under 35 U.S.C. § 112

Claims 1-9, 15 and 16 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. New independent claims were written to particular point out and distinctly claim the subject matter of the invention. The rejected dependent claims now depend on the new independent claims.

### Rejection under 35 U.S.C. § 102(b)

Claims 1-6, 15 and 16 were rejected under 35 U.S.C. § 102(b) as being anticipated by Jorgensen et al. (US 2003/0175205).

The invention of Jorgensen et al. relates to using lipid-based composition to target diagnostic agents (i.e., “labels”). The Jorgensen disclosure is useful in the diagnosis of various disorders which are associated with or resulting from increased levels of extracellular PLA<sub>2</sub> activity in the diseased tissue, e.g., cancer, infections and inflammatory conditions (see Abstract of Jorgensen, US 2003/0175205). In particular, the Jorgensen publication is directed to image enhancing systems. The dye is released in order to test the system, to see if PLA<sub>2</sub> from, for example, a tumor might release the imaging label in vivo. The liposome system of Jorgensen is never used to test the presence or strength of PLA<sub>2</sub>

PLA<sub>2</sub> are commonly found in mammalian tissues as well as insect and snake venom. Venom from both snakes and insects is largely composed of melittin which is a stimulant of PLA<sub>2</sub>. Due to the increased presence and activity of PLA<sub>2</sub> resulting from a snake or insect bite, arachidonic acid is released from the phospholipid membrane disproportionately. As a result, inflammation and pain occur at the site. (See attached Wikipedia entry.)

In contrast, the present invention relates to a method of screening for compounds or salts thereof that are safe for gastric mucosa and causes little gastrointestinal side effects. The present invention has no relation with the localized activity of extracellular PLA<sub>2</sub> activity. A person skilled in the art of gastric safety of new drugs would not be motivated by a disclosure concerning “tumor imagining with contrast liposomes” (see [0012] of the Jorgensen reference.) Nothing in Jorgensen would describe or suggest a comparative drug screen as delineated in the new claims. It is only by using the specification of the present application and impermissible hindsight that one can seek the calcein containing liposomes in the Jorgensen reference.

In addition, given the unpredictability of the biological arts, there would be no way to know that the membrane model system of applicants would actually work to predict gastric safety. The validation experiments of examples 2 and 3 show that the greater leakage of fluorescent dye in the method of invention in fact correlates with NSAIDs having greater toxicity demonstrating that the method is accurate and useable.

Before the validation experiments, it could have easily turned out that there was no correlation.

**Rejection under 35 U.S.C. § 103(a)**

Claims 7-9 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Jorgensen et al. (US 2003/0175205).

As mentioned above, the present invention has no relation with the localized activity of extracellular PLA<sub>2</sub> activity as disclosed in Jorgensen et al. The present invention related to a method of screening for compounds or salts thereof that are safe for gastric mucosa and causes little gastrointestinal side effects. The present invention provides a means for determining the degree of damage of phospholipid liposome membrane when it is allowed to react with a given test compound; in other words, the claimed method uses the phospholipid liposome membrane as an analogue of gastric mucosa. The need for validation would be particularly crucial to show that the method works to screen for a compound that may be protective as described in claim 9 and new claim 19. All of the other arguments presented in connection with the 102(b) rejection are incorporated herein.

These specific characteristics of the applicants' invention are not described or suggested in Jorgensen et al. Consequently, the person skilled in the art does not have the knowledge or motivation to make the present invention. Applicants respectfully submit that the present claims are not obvious in light of Jorgensen et al. and are patentable.

Application No. 10/597,483  
Amendment dated May 26, 2009  
Reply to Non-Final Office Action of Feb 26, 2009

## CONCLUSION

If the Examiner has any questions or suggested Examiner's amendments, the Examiner is respectfully requested to call the undersigned.

The Commissioner is hereby authorized to charge any additional fees, or to credit any overpayment, to Deposit Account No. 50-3195.

Respectfully submitted,

Date: May 26, 2009

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Appendix  
1. PLA<sub>2</sub> Wikipedia Entry

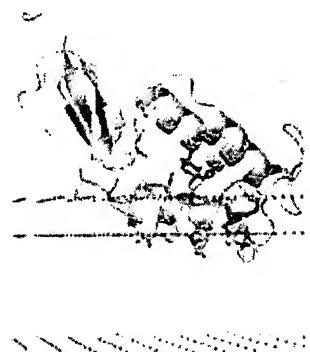
# Phospholipase A2

From Wikipedia, the free encyclopedia

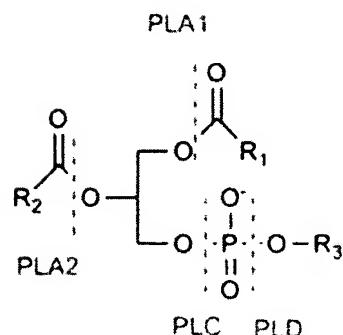
**Phospholipases A2 (PLA2s) EC 3.1.1.4** (<http://www.expasy.org/cgi-bin/nicezyme.pl?3.1.1.4>) are upstream regulators of many inflammatory processes. This particular phospholipase specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing arachidonic acid and lysophospholipids. Upon downstream modification by cyclooxygenases, arachidonic acid is modified into active compounds called eicosanoids. Eicosanoids include prostaglandins and leukotrienes which are categorized as inflammatory mediators.<sup>[1]</sup>

PLA2 are commonly found in mammalian tissues as well as insect and snake venom.<sup>[2]</sup> Venom from both snakes and insects is largely composed of melittin which is a stimulant of PLA2. Due to the increased presence and activity of PLA2 resulting from a snake or insect bite, arachidonic acid is released from the phospholipid membrane disproportionately. As a result, inflammation and pain occur at the site.<sup>[3]</sup> There are also prokaryotic A2 phospholipases.

Additional types of phospholipases include phospholipase A1, phospholipase B, phospholipase C, and phospholipase D.<sup>[4]</sup>



**Bee venom phospholipase A2**  
sPLA2. Middle plane of the lipid bilayer - black dots. Boundary of the hydrocarbon core region - red dots (extracellular side). Layer of lipid phosphates - yellow dots.



**Phospholipase Cleavage Sites.**  
Note that an enzyme that displays both PLA1 and PLA2 activities is called a *Phospholipase B*

## Contents

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- 3 Regulation
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## Families

Phospholipases A2 include several unrelated protein families with common enzymatic activity. Two most notable families are secreted and cytosolic phospholipases A2. Other families include Ca<sup>2+</sup> independent PLA2 (iPLA2) and lipoprotein-associated PLA2s (Ip-PLA2), also known as platelet activating factor acetylhydrolase (PAF-AH).

## Secreted phospholipases A2 (sPLA2)

The extracellular forms of phospholipases A2 have been isolated from different venoms (snake, bee, and wasp), from virtually every studied mammalian tissue (including pancreas and kidney) as well as from bacteria. They require  $\text{Ca}^{2+}$  for activity.

Pancreatic PLA2 serve for the initial digestion of phospholipid compounds in dietary fat. Venom phospholipases help to immobilize prey by promoting cell lysis.

## Cytosolic phospholipases A2 (cPLA2)

The intracellular PLA2 are also Ca-dependent, but they have completely different 3D structure and significantly larger than secreted PLA2 (more than 700 residues). They include C2 domain and large catalytic domain.

These phospholipases are involved in cell signaling processes, such as inflammatory response. The produced Arachidonic acid is both a signaling molecule and the precursor for other signalling molecules termed eicosanoids. These include leukotrienes and prostaglandins. Some eicosanoids are synthesized from diacylglycerol, released from the lipid bilayer by phospholipase C (see below).

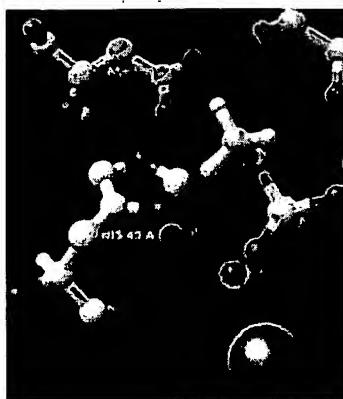
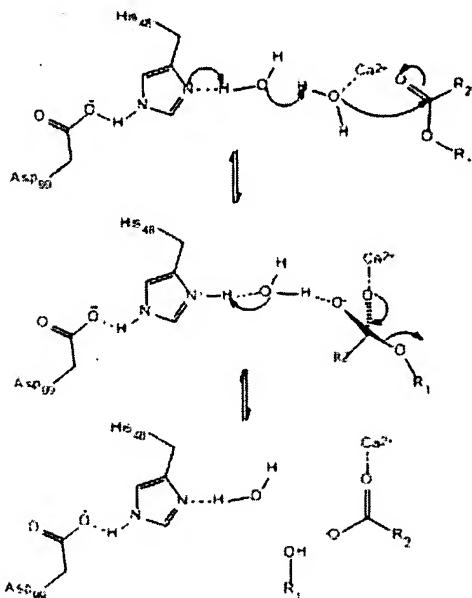
Phospholipases A2 can be classified based on sequence homology.<sup>[5]</sup>

## Lipoprotein-associated PLA2s (lp-PLA2)

Increased levels of lp-PLA2 are associated with cardiac disease, and may contribute to atherosclerosis<sup>[6]</sup>.

## Mechanism

The suggested catalytic mechanism of pancreatic sPLA2 is initiated by a His-48/Asp-99/calcium complex within the active site. The calcium ion polarizes the sn-2 carbonyl oxygen while also coordinating with a catalytic water molecule, w5. His-48 improves the nucleophilicity of the catalytic water via a bridging second water molecule, w6. It has been suggested that two water molecules are necessary to traverse the distance between the catalytic histidine and the ester. The basicity of His-48 is thought to be enhanced through hydrogen bonding with Asp-99. An asparagine substitution for His-48 maintains wild-type activity, as the amide functional group on asparagine can also function to lower the pKa, or acid dissociation constant, of the bridging water molecule. The rate limiting state is characterized as the degradation of the tetrahedral intermediate composed of a calcium coordinated oxyanion. The role of calcium can also be duplicated by other relatively small cations like cobalt and nickel. [8]



Close-up rendering of PLA2 active site with phosphate enzyme inhibitor. Calcium ion (pink) coordinates with phosphate (light blue).

Phosphate mimics tetrahedral intermediate blocking substrate access to active site. His-48, Asp-99, and 2 water molecules are also shown. [7][1] (<http://www.pdb.org/pdb/explore/explore.do?structureId=1FXF>) From PDB 1FXF (<http://www.rcsb.org/pdb/explore/explore.do?structureId=1FXF>)

PLA2 can also be characterized as having a channel featuring a hydrophobic wall in which hydrophobic amino acid residues such as Phe, Leu, and Tyr serve to bind the substrate. Another component of PLA2 is the seven disulfide bridges which are influential in regulation and stable protein folding. [8]

## Regulation

Due to the importance of PLA2 in inflammatory responses, regulation of the enzyme is essential. PLA2 is regulated by phosphorylation and calcium concentrations. PLA2 is phosphorylated by a MAPK at Serine-505. When phosphorylation is coupled with an influx of calcium ions, PLA2 becomes stimulated and can translocate to the membrane to begin catalysis. [9]

Phosphorylation of PLA2 may be a result of ligand binding to receptors, including:

Phospholipase A2	
<b>Identifiers</b>	
<b>Symbol</b>	Phospholip_A2_1
<b>Pfam</b>	PF00068 ( <a href="http://pfam.sanger.ac.uk/family?acc=PF00068">http://pfam.sanger.ac.uk/family?acc=PF00068</a> )
<b>InterPro</b>	IPR001211 ( <a href="http://www.ebi.ac.uk/interpro/DisplayIproEntry?ac=IPR001211">http://www.ebi.ac.uk/interpro/DisplayIproEntry?ac=IPR001211</a> )

- 5-HT2 receptors<sup>[10]</sup>
- mGLUR1<sup>[10]</sup>
- bFGF receptor<sup>[10]</sup>
- INF- $\alpha$  receptor<sup>[10]</sup>
- INF- $\gamma$  receptor<sup>[10]</sup>

## Relevance in Neurological Disorders

In normal brain cells, PLA2 regulation accounts for a balance between arachidonic acid conversion into proinflammatory mediators and arachidonic acid reincorporation into the membrane. In the absence of strict regulation of PLA2 activity, a disproportionate amount of proinflammatory mediators are produced. The resulting induced oxidative stress and neuroinflammation is analogous to neurological diseases such as Alzheimer's disease, epilepsy, multiple sclerosis, ischemia. Lysophospholipids are another class of molecules released from the membrane that are upstream predecessors of platelet activating factors (PAF). Abnormal levels of potent PAF are also associated with neurological damage. An optimal enzyme inhibitor would specifically target PLA2 activity on neural cell membranes already under oxidative stress and potent inflammation. Thus, specific inhibitors of brain PLA2 could be a pharmaceutical approach to treatment of several disorders associated with neural trauma.<sup>[11]</sup>

Increase in phospholipase A2 activity is an acute phase reaction that rises during inflammation, which is also seen to be exponentially higher in low back disc herniations compared to rheumatoid arthritis. It is a mixture of inflammation and substance P that are responsible for pain.

Increased phospholipase A2 has also been associated with neuropsychiatric disorders such as schizophrenia and pervasive developmental disorders (such as autism), though the mechanisms involved are not known.<sup>[12]</sup>

## Human proteins containing phospholipase A2 domain

OC90; PLA2G10; PLA2G1B; PLA2G2A; PLA2G2D; PLA2G2E; PLA2G2F; PLA2G3; PLA2G5;

## References

1. ^ Dennis, et al. "Diversity of Group Types, Regulation, and

### PROSITE PDOC00109

(<http://www.expasy.org/cgi-bin/prosite-search-ac?PDOC00109>)

**SCOP** 1bbc (<http://scop.mrc-lmb.cam.ac.uk/scop/search.cgi?tlev=fa;&pdb=1bbc>)  
**OPM family** 90 (<http://opm.phar.umich.edu/families.php?superfamily=90>)  
**OPM protein** 1g4i (<http://opm.phar.umich.edu/protein.php?search=1g4i>)

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Function of Phospholipase A2." *The Journal of Biological Chemistry*. Vol. 269. No 18. 13057-13060.

2. ^ Nicholas, et al. "Localization of Structural Elements of Bee Venom Phospholipase A2 Involved in N-type Receptor Binding and Neurotoxicity." *Journal of Biological Chemistry*. Vol. 227. No. 11. 7173-7181.
3. ^ Argiolas, Pianto. "Facilitation of Phospholipase A2 Activity by Mastoparans, a New Class of Mast Cell Degranulating Peptides from Wasp Venom." *Journal of Biological Chemistry*. Vol. 285. Issue 122. 13697-13702
4. ^ Lehninger. "Principles of Biochemistry". 2004. W H Freeman and Company. Fourth Edition.
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7. ^ Pan, Y.H., Epstein, T.M., Jain, M.K., Bahnsen, B.J. (2001) Five coplanar anion binding sites on one face of phospholipase A2: relationship to interface binding. *Biochemistry* 40: 609-617
8. ^ <sup>a b</sup> Berg et al. "Interfacial Enzymology: The Secreted Phospholipase A2-Paradigm" *Chemical Review*, 2001. Vol. 101. No. 9 2638-2640.
9. ^ Leslie et al. "Properties and Regulation of Cytosolic Phospholipase A2". 1997. *The Journal of Biochemistry*. Vol. 272. No. 27. 16709-16712.
10. ^ <sup>a b c d e</sup> Walter F., PhD. Boron (2003). *Medical Physiology: A Cellular And Molecular Approach*. Elsevier/Saunders. p. 1300. ISBN 1-4160-2328-3. Page 103
11. ^ Farooqui et al. "Inhibitors of Brain Phospholipase A2 Activity: Their Neuropharmacological Effects and Therapeutic Importance for the Treatment of Neurologic Disorders" 2006. *Pharmacological Reviews*. Vol. 58. 591-620.
12. ^ Bell, JG et al.. Essential fatty acids and phospholipase A2 in autistic spectrum disorders. 2004. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. Vol. 71. 201-204

## Genes

- Group I: *PLA2G1B* ([http://www.genenames.org/data/hgnc\\_data.php?match=PLA2G1B](http://www.genenames.org/data/hgnc_data.php?match=PLA2G1B))
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- Group VII: *PLA2G7* ([http://www.genenames.org/data/hgnc\\_data.php?match=PLA2G7](http://www.genenames.org/data/hgnc_data.php?match=PLA2G7))
- Group X: *PLA2G10* ([http://www.genenames.org/data/hgnc\\_data.php?match=PLA2G10](http://www.genenames.org/data/hgnc_data.php?match=PLA2G10))
- Group XII: *PLA2G12A* ([http://www.genenames.org/data/hgnc\\_data.php?match=PLA2G12A](http://www.genenames.org/data/hgnc_data.php?match=PLA2G12A)) , *PLA2G12B* ([http://www.genenames.org/data/hgnc\\_data.php?match=PLA2G12B](http://www.genenames.org/data/hgnc_data.php?match=PLA2G12B))

## External links

- Phospholipase A2 active sites (<http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00109>) in PROSITE
- UMich Orientation of Proteins in Membranes *families/superfamily-90* (<http://opm.phar.umich.edu/families.php?superfamily=90>) - Secreted phospholipases A2 in the lipid bilayer
- UMich Orientation of Proteins in Membranes *families/superfamily-134* (<http://opm.phar.umich.edu/families.php?superfamily=134>) - Cytosolic phospholipase A2 and patatin
- MeSH *Phospholipase+A2* ([http://www.nlm.nih.gov/cgi/mesh/2009/MB\\_cgi?mode=&term=Phospholipase+A2](http://www.nlm.nih.gov/cgi/mesh/2009/MB_cgi?mode=&term=Phospholipase+A2))

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